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Effects of tissue plasminogen activator timing on blood–brain barrier permeability and hemorrhagic transformation in rats with transient ischemic stroke

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ABSTRACT

The goal of our study was to determine if the timing of the tissue plasminogen activator (tPA) administration influenced its effect on blood–brain barrier (BBB) permeability and the subsequent risk of hemorrhagic transformation.

Thirty spontaneously hypertensive male rats were subjected to a 90-minute unilateral middle cerebral artery occlusion. Six rats did not receive tPA treatment (vehicle control: Group 0), intravenous tPA was administered immediately after reperfusion (Group 1) or 4 h after reperfusion (Group 2). Dynamic contrast enhancement (DCE) and gradient-echo (GRE) MR sequences were used to assess the dynamic evolution of BBB permeability and hemorrhagic transformation changes at the following time points: during occlusion, and 3 h, 6 h, and 24 h post reperfusion.

In all groups, BBB permeability values in the ischemic tissue were low during occlusion. In Group 0, BBB permeability values increased at 3 h after reperfusion ($p = 0.007$, compared with the values during occlusion), and further at 6 h after reperfusion ($p = 0.004$, compared with those at 3 h post reperfusion). At 24 h post reperfusion, the values decreased to a level relative to but still higher than those during occlusion ($p = 0.025$, compared with the values during occlusion). At 3 h after reperfusion, BBB permeability values in the ischemic tissue increased, but to a greater extent in Group 1 than in Group 0 ($p = 0.034$) and Group 2 ($p = 0.010$). At 6 h after reperfusion, BBB permeability values in the ischemic tissue increased further in Group 2 than in Group 0 ($p = 0.006$) and Group 1 ($p = 0.001$), while Group 1 exhibited BBB permeability that were still abnormal but less than those observed at 3 h ($p = 0.001$). Group 2 tended to have a higher hemorrhage incidence (36.4%, 4/11) than Group 1 (10.0%, 1/10, $p = 0.311$) and Group 0 (0%), and hemorrhages occurred around 6 h after reperfusion when BBB permeability values were the highest. Mortality was higher in Group 2 (63.6%, 7/11) than in Group 0 (0%) and Group 1 (10.0%, 1/10, $p = 0.024$).

The findings suggest that the timing of tPA administration is of importance for its impact on BBB permeability and subsequent risk of hemorrhagic transformation.

1. Introduction

Hemorrhagic transformation (HT) is a feared complication of acute ischemic stroke. It can arise as the result of an ischemic damage to the

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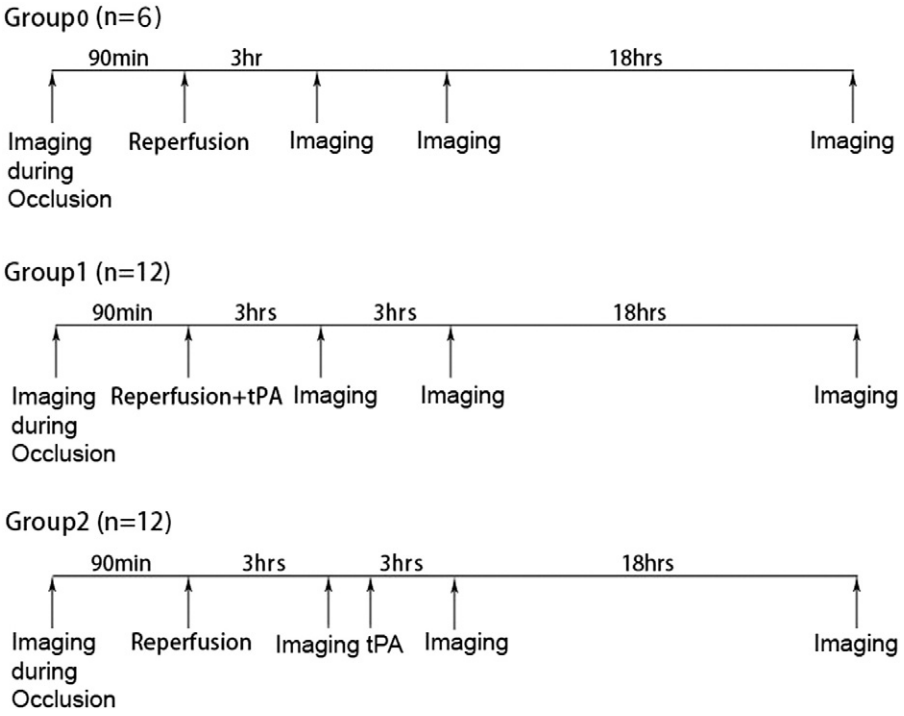

Fig. 1. Schematic representation of our study design and study groups. Group 0: vehicle control, without tPA administration. Group 1: tPA was administered immediately after reperfusion. Group 2: tPA was administered after the MR imaging at 3 h post reperfusion, and the imaging lasted approximately 1 h.

blood–brain barrier (BBB) with subsequent vascular leakage [7,26]. It is often triggered by reperfusion [32].

Tissue plasminogen activator (tPA) has been shown to be a successful thrombolytic drug in acute ischemic stroke patients [11,12] but significantly increases the risk of symptomatic HT [11,33,35]. In the National Institute of Neurological Disorders and Stroke (NINDS) tPA trial [1], the percentage of tPA-treated patients who developed significant HT following an ischemic stroke was 6.4% as compared with just 0.6% in the placebo group. One of the mechanisms by which tPA causes HT is its impact on the BBB permeability [20,27,32].

Reperfusion injury resulting from the thrombolytic effect of tPA involves reactive oxygen species and oxidative stress, which degrade protein and lipid components vital to the BBB function [14]. Independently of reperfusion, tPA activates matrix metalloproteinases (MMPs), which in turn alter the basal lamina of vascular endothelium, weaken vessels, and favors leakage and rupture [2,5,6,13,16,24,27,29,31,32].

The BBB permeability changes evolve in a dynamic process after reperfusion. In a 2-hour temporary middle cerebral artery occlusion (MCAO) model, Belayev et al. explored the time course and regional pattern of blood–brain barrier (BBB) opening after reperfusion, the quantitation of Evans' blue extravasation indicated some degree of BBB disruption occurring at 3–4 h after MCAO, maximal disruption at 5 h, and delayed BBB disruption at 48–50 h [3]. In a study with 3-h MCAO, the water content accumulated with time in the ipsilateral hemisphere within 12 h post MCAO (Slivka et al., 1995) [36]. In our study, we wanted to focus on the direct effect of tPA on the BBB permeability and on the risk of hemorrhagic transformation. We thus assessed the impact of tPA injected at various timepoints, while maintaining the duration of the occlusion constant.

2. Materials and methods

2.1. Study design

This animal study was approved by the Institutional Animal Care and Use Committee of the University of Virginia (Charlottesville, VA). All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Spontaneously hypertensive (SHR) male rats (Charles River Laboratories, Inc., Wilmington, MA.), body weight 260–280 g, 11–13 weeks old, were subjected to 90 min of transient focal cerebral ischemia. The animals were then randomly assigned to one of the three treatment groups: (1) Group 0, controls: the rats were administered the same dose of saline vehicle as that of tPA (10 mg/kg, 10% administered as a bolus and the remainder as a continuous infusion over 60 min), (2) Group 1: tPA administered immediately after reperfusion, and (3) Group 2: tPA administered 4 h after reperfusion (Fig. 1). To determine whether the results would be influenced by the type of stroke model, we used two techniques to produce transient focal cerebral ischemia: (1) 3 vessel-occlusion technique (3VO) and (2) intraluminal filament occlusion technique (fMCAO).

Recombinant human tPA (Genentech, San Francisco, CA, USA) was reconstituted with the concentration of 1 mg/ml, and infused intravenously via the tail vein at a dose of 10 mg/kg. Ten percent was administered as a bolus and the remainder as a continuous infusion over 60 min, using a syringe infusion pump (New era pump system, Inc, Famingdale, NY, USA). This relatively high dosage was used because there is an approximately 10-fold difference in fibrin-specific enzyme activity between humans and rodents [23,31].

MR imaging was obtained at four time points: during occlusion, and at 3 h, 6 h, and 24 h post reperfusion. The MRI during occlusion and 3 h after reperfusion were used to confirm the inclusion and exclusion of the animals in the study. Exclusion criteria included: the diffusion-weighted imaging lesion during occlusion was too small (involved only subcortical regions, but not the cortex); the imaging before tPA administration (during occlusion or 3 h after reperfusion) showed intracranial hemorrhage.

2.2. Animal surgery

All surgery and MRI were conducted on the animals under anesthesia, which was induced by isoflurane at 4% and then maintained at 2%. During the procedures, the rats were intubated and ventilated